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DNA methylation and cognitive functioning in healthy older adults

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ABSTRACT

Long-term supplementation with folic acid may improve cognitive performance in older individuals. The relationship between folate status and cognitive performance might be mediated by changes in methylation capacity, as methylation reactions are important for normal brain functioning. Although aberrant DNA methylation has been implicated in neurodevelopmental disorders, the relationship between DNA methylation status and non-pathological cognitive functioning in humans has not yet been investigated. The present study investigated the associations between global DNA methylation and key domains of cognitive functioning in healthy older adults. Global DNA methylation, defined as the percentage of methylated to total cytosine, was measured in leukocytes by LC-MS/MS, in 215 men and women, aged 50-70 years, who participated in the FACIT study (clinical trial registration number NCT00110604). Cognitive performance was assessed by means of the Visual Verbal Word Learning Task, the Stroop Colour-Word Interference Test, the Concept Shifting Test, the Letter-Digit Substitution Test, and the Verbal Fluency Test. Using hierarchical linear regression analyses adjusted for age, sex, level of education, alcohol consumption, smoking status, physical activity, erythrocyte folate concentration, and *MTHFR* 677C→T genotype, global DNA methylation was not related to cognitive performance on any of the domains measured. Our results do not support the hypothesis that global DNA methylation, as measured in leukocytes, might be associated with cognitive functioning in healthy older individuals.

Introduction

Most cognitive functioning declines with advancing age, and identifying the risk factors for age-related cognitive decline has become a topic of increasing interest. Previous research has indicated that a low folate status might increase the risk of cognitive impairment[1]. However, the potential biological mechanisms underlying this relationship remain to be elucidated.

One possible mechanism that might explain the involvement of folate status in cognitive performance is DNA methylation, which refers to the epigenetic modification of gene expression by the addition of methyl groups to cytosine residues in DNA[2]. Recent animal studies have suggested that DNA methylation may be involved in regulating synaptic plasticity in hippocampal neurons, thereby influencing learning and memory processes[3,4]. In humans, both hypomethylation and hypermethylation of DNA have been implicated in psychiatric disorders, including schizophrenia[5], neurodegenerative disorders, such as Alzheimer's disease[6], and syndromes associated with mental retardation, e.g. Fragile X syndrome[7].

Methyl groups for DNA methylation are provided by the universal methyl donor *S*-adenosylmethionine, which is synthesized from methionine[8]. Folic acid may increase the availability of *S*-adenosylmethionine by promoting the conversion of homocysteine into methionine, thereby influencing DNA methylation status[9]. Indeed, an intervention study in older women has shown that low dietary folate intake was associated with global DNA hypomethylation, which could be reversed by folate repletion[10]. In addition, the common *MTHFR* 677C→T polymorphism, which mimics folate deficiency by impairing the conversion of homocysteine into methionine, has also been related to DNA hypomethylation[11].

Given the role of folate metabolism in generating methyl donors for methylation processes, and the involvement of DNA methylation in brain functioning, it seems reasonable to hypothesize that folate status might influence cognitive functioning by exerting effects on DNA methylation. However, the association between DNA methylation status and cognitive performance in the general population has not yet been investigated. Therefore, the present study examined whether

leukocyte global DNA methylation was associated with cognitive performance in healthy older adults.

Methods

Study population

The present study was performed using data from the FACIT study, a randomized, double-blind, placebo-controlled trial, originally designed to investigate the effects of 3-year folic acid supplementation on the risk of cardiovascular disease[12]. The study population consisted of 818 healthy men and women, aged 50-70 years at baseline. A detailed description of the study design and the selection of participants can be found elsewhere[12].

Venous blood samples were collected at baseline. Leukocyte global DNA methylation was determined in a subsample of 216 participants. First, the study population was stratified by *MTHFR* 677C→T genotype, to ensure equal distribution of *MTHFR* 677C→T genotypes in the final sample. Thereafter, participants in the folate treatment group were randomly selected from the three strata and individually matched with participants in the placebo group on the variables age, sex, smoking status, and *MTHFR* 677C→T genotype, as these variables may influence DNA methylation[11,13,14]. Some samples were not measured due to human error in sample retrieval. Valid DNA methylation data were available for 111 participants in the treatment group and 105 participants in the placebo group. As valid data on cognitive functioning were lacking for one participant in the folate treatment group, the final study sample consisted of 215 individuals.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human participants were approved by the Medical Ethics Committee of Wageningen University. Written informed consent was obtained from all participants.

Cognitive functioning

Cognitive functioning on the domains of memory, sensorimotor speed, complex speed, information processing speed, and word fluency was assessed by means of a comprehensive neuropsychological test battery, consisting of the Visual Verbal Word Learning Task, the Stroop

Colour-Word Interference Test, the Concept Shifting Test, the Letter-Digit Substitution Test, and the Verbal Fluency Test, as described before[12].

DNA methylation status and genotyping

Genomic DNA was isolated from peripheral blood leukocytes at baseline. Global DNA methylation was determined by LC-MS/MS, as described previously[15]. Genomic DNA methylation status was calculated as the percentage of methylated to total cytosine (mCyt/tCyt) using the following formula: (nmol mCyt/[nmol mCyt + nmol Cyt]) × 100%[15].

MTHFR 677C→T genotype was determined by PCR with restriction fragment length polymorphism analysis with *Hin*fI[16], and was defined as common variant (CC or CT genotype) or rare variant (TT genotype).

Blood measurements

Fasting venous blood samples were collected at baseline, directly processed, and stored at -80°C. Serum folate was measured using a chemiluminescent immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). Erythrocyte folate was determined in duplicate and the average was taken to reduce measurement error. Erythrocyte folate concentrations were calculated using the following formula: (unadjusted erythrocyte folate/hematocrit) – ([1 – hematocrit]/hematocrit) × serum folate. Plasma total homocysteine was determined by HPLC and fluorimetric detection, as described previously[17].

Demographic and lifestyle variables

Level of education (low/middle/high) was measured by classifying formal schooling according to the Dutch educational system[18]. Alcohol consumption (g/d) and current smoking (yes/no) were ascertained by means of self-report questionnaires. BMI (kg/m^2) was calculated from height and weight, and physical activity was estimated using the Physical Activity Scale for the Elderly[19].

Statistical analysis

Normality of data distributions was ascertained by normal P-P plots. Baseline data were used to assess the cross-sectional associations between total DNA methylation status and cognitive functioning. Independent samples *t* tests and univariate ANOVA were used to examine whether DNA methylation status varied according to sex, level of education, smoking status, or *MTHFR* 677C→T genotype.

Hierarchical linear regression analyses were performed for DNA methylation status in relation to each of the five cognitive performance indices. The analyses were corrected for sociodemographic and lifestyle variables that were considered potential confounders, i.e. age, sex, level of education, alcohol consumption, smoking status, physical activity, erythrocyte folate concentration, and *MTHFR* 677C→T genotype[11,13,14,20].

To investigate the possibility of a non-linear relationship between global DNA methylation and cognitive performance, the analyses were repeated with the quadratic term for DNA methylation status as the independent variable, adjusted for covariates and the linear term for DNA methylation status. The quadratic term for DNA methylation status was expressed as the residuals of regressing (DNA methylation)^2 on DNA methylation, i.e. the quadratic component that is orthogonal to the linear component of DNA methylation.

Statistical power for detecting associations between DNA methylation status and each of the dependent variables, assuming a small effect size of $f^2 = 0.03$, was 0.80. Statistical differences were considered significant at *P*-values <0.05. All analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 summarizes the characteristics of the study population. The percentage of methylated to total cytosine residues in leukocyte DNA ranged from 4.0 to 5.6%, which was comparable to the range reported by other population-based studies[11,15]. The extent of global DNA methylation did not vary according to sex (t = -1.285, P = 0.200), level of education (F = 0.611, P = 0.544), smoking status (t = 1.611, P = 0.109), or *MTHFR* 677C→T genotype (t = -0.907, P = 0.365).

Hierarchical linear regression analyses corrected for age, sex, level of education, alcohol consumption, smoking status, physical activity, erythrocyte folate concentration, and *MTHFR* 677C→T genotype did not reveal any significant associations between leukocyte global DNA methylation and cognitive performance on any of the domains measured (Table 2). In addition, repeating the analyses with the quadratic term for DNA methylation status as the independent variable did not yield any significant results (data not shown), implying that global DNA methylation did not show a non-linear relationship with cognitive performance.

Discussion

The present study did not offer support for the hypothesis that individual variation in cognitive functioning in older adults might be related to the extent of leukocyte global DNA methylation.

Although there are no previous studies investigating the relationship between global DNA methylation and cognitive functioning in healthy humans, aberrant DNA methylation has been implicated in neurodevelopmental disorders[7], psychiatric diseases[5], and neurodegenerative disorders[6]. In addition, animal research has suggested that DNA methylation status may be involved in learning and memory processes, e.g. by regulating synaptic plasticity in hippocampal neurons[3,4].

The observed lack of a relationship between global DNA methylation and cognitive performance in healthy adults might imply that there is no functional relationship between the extent of cytosine methylation within DNA and individual differences in cognitive performance in the general population. In line with earlier reports[15], we observed that global DNA methylation has a relatively narrow distribution in healthy individuals. These findings suggest that under non-pathological conditions, there appears to be little interindividual variation in DNA methylation-based regulation of gene expression, which decreases the likelihood that individual differences in cognitive performances may be mediated by this epigenetic mechanism.

Although global DNA methylation might not be involved in cognitive functioning, the present results do not rule out the possibility that DNA methylation at specific loci may be related to cognitive performance. In humans, gene-specific alterations in DNA methylation patterns have been associated with a number of pathological conditions characterized by cognitive deficits. Animal studies have suggested that diet-induced folate deficiency may result in overexpression of the Presenilin 1 gene by causing hypomethylation of its promoter region[21]. Increased expression of this gene, which leads to elevated production of β-amyloid peptide, has been implicated in the etiology of Alzheimer's disease[22]. In addition, schizophrenia has been associated with reduced expression of the gene encoding the protein Reelin, which is involved in neurodevelopment and synaptic plasticity, due to hypermethylation of the gene's promoter region[5]. However, although it

may be speculated that gene-specific changes in DNA methylation might underlie part of the individual differences in non-pathological cognitive functioning, little is known about the genetic correlates of cognitive performance in healthy humans.

An alternative explanation for the present null findings is that cognitive performance might be related to short-term changes, i.e. within the range of hours, in DNA methylation patterns rather than individual variation on the level of global DNA methylation. Indeed, animal studies have reported that dynamic and reversible changes in DNA methylation, such as the transient methylation and demethylation of DNA, are crucial for synaptic plasticity, learning, and memory processes[3,4]. It might be complicated, however, to measure such short-term changes in DNA methylation in volunteers, which makes it rather difficult to test this possibility.

From a methodological perspective, our study was limited by its cross-sectional nature. In addition, the fact that we determined global DNA methylation in leukocytes rather than brain tissue should also be considered a limitation, as the extent of DNA methylation might differ between cells derived from the periphery and the brain[23]. However, no direct measures of DNA methylation status in the central nervous system were available, given the inability to measure cerebrospinal fluid or brain DNA methylation status in volunteers.

It might also be argued that due to the relatively small sample size, the present study might have been underpowered to detect very modest associations. However, it should be noted that our study had 80% power to detect a 3% change in the proportion of explained variance, which may be considered a small effect size[24].

The present study did not support the notion that folate metabolism might influence cognitive performance through the mechanism of global DNA methylation, as measured in leukocytes. In line with the present findings, we found that long-term supplementation with folic acid, which significantly improved cognitive performance in the FACIT population[12], did not have any effect on leukocyte global DNA methylation status (A. Jung, Y. Smulders, P. Verhoef, F.J. Kok, H. Blom, R. Kok, E. Schouten, E. Kampman, J. Durga, 2010; unpublished results). This might

be explained by the fact that methylation capacity is not exclusively dependent on folate status, as methyl groups may also be provided by dietary intake of methionine, or by betaine-mediated remethylation of homocysteine[9].

To our knowledge, this is the first study to investigate the relationship between leukocyte global DNA methylation and non-pathological cognitive functioning in healthy older adults. Future studies focusing on gene-specific DNA methylation patterns or short-term changes in DNA methylation status might contribute further to identifying the epigenetic mechanisms involved in cognitive functioning.

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Table 1. Characteristics of the study population.

Characteristic	Total sample (n = 215)	95% CI
Age (years)	60.9	60.2; 61.6
Female sex (%)	34.9	
Level of education (% low / middle / high)	26.0 / 39.1 / 34.9	
Alcohol consumption (g/d)*	12.6	4.5; 23.5
Current smoker (%)	14.9	
BMI (kg/m^2)	26.7	26.2; 27.2
Physical activity (PASE score)	149.2	140.5; 158.0
Erythrocyte folate (nmol/l)	716.0	681.2; 750.8
Plasma total homocysteine (μmol/l)	13.4	12.9; 13.8
MTHFR 677C→T genotype (% CC / CT / TT)	34.9 / 32.6 / 32.6	
Leukocyte global DNA methylation status (%)†	4.6	4.6; 4.7

Values are means or %. PASE, Physical Activity Scale for the Elderly; *MTHFR*, 5,10-methylenetetrahydrofolate reductase.

* Median (interquartile range) is given because of skewed data distribution.

† Defined as the percentage of methylated to total cytosine (mCyt/tCyt).